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Importance of the hydrogen route in up-scaling electrosynthesis for microbial CO_2 reduction

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Broader context

In the context of CO_2 conversion to fuels and chemicals, the association of electrochemistry with microbial catalysis has opened up promising new routes to reduce CO_2 to acetate and other multi-carbon compounds. The present paper points out that some of these electro-microbial processes are based on two consecutive steps: firstly the electrochemical production of hydrogen by water electrolysis and, secondly, the reduction of CO_2 by microbial species that use the hydrogen produced. In consequence, the implementation of homoacetogenic microorganisms with direct CO_2 and hydrogen gas supply should now be considered as a worthwhile strategy for CO_2 conversion. Hydrogen can be produced in optimal conditions by conventional electrolysis, preferentially fed with electrical energy harvested using renewable strategies, and then used to drive CO_2 conversion in an H_2-CO_2 gas-liquid bioreactor.

Abstract

Microbial electrochemical reduction of CO_2 was carried out under two different applied potentials, -0.36 V and -0.66 V vs. SHE, using biological sludge as the inoculum. Both potentials were thermodynamically appropriate for converting CO_2 to acetate but only -0.66 V

enabled hydrogen evolution. No acetate production was observed at -0.36 V, while up to 244 ± 20 mg/L acetate was produced at -0.66 V vs. SHE. The same microbial inoculum implemented in gas-liquid contactors with H_2 and CO_2 gas supply led to acetate production of 2500 mg/L. When salt marsh sediment was used as the inoculum, no reduction was observed in the electrochemical reactors, while supplying H_2+CO_2 gas led to formate and then acetate production. Finally, pure cultures of *Sporomusa ovata* grown under H_2 and CO_2 gas feeding showed acetate production of up to 2904 mg/L, higher than reported so far in the literature for *S. ovata* implemented in bioelectrochemical processes. Unexpected ethanol production of up to 1411 mg/L was also observed. All these experimental data confirm that hydrogen produced on the cathode by water electrolysis is an essential mediator in the microbial electrochemical reduction of CO_2. Implementing homoacetogenic microbial species in purposely designed gas-liquid biocontactors should now be considered as a relevant strategy for developing CO_2 conversion.

Keywords: Carbon dioxide, electrochemical reduction, microbial electrosynthesis, bioelectrochemical system, hydrogen.

1. INTRODUCTION

Bulk chemicals and liquid fuels are currently produced almost exclusively from petrochemical feedstock. In the light of emission reduction targets, the production of chemicals from CO_2 or other renewable resources may play an important role in decreasing our environmental impact. There are several advantages to using CO_2 as a reactant, such as unlimited availability (atmosphere, waste gas, etc.), land-independence, ease of handling and limited toxicity [1]. In this framework, electrochemical processes offer various options for converting CO_2 to fuels and commodities. Reducing CO_2 by electrochemistry is a way of converting electrical energy harvested with renewable strategies, such as solar or wind, into chemical forms that can be stored and then distributed on demand. Various electro-catalysts have been designed with some success for the reduction of CO_2 to methanol or formate [2], including enzymes [3]. More specific attempts have also been reported, including the reduction to H_2+CO syngas mixtures [4] and even reduction to carbon by molten salt electrolysis [5].

Over the past decade, microbial electrosynthesis has emerged as an additional option for the electro-reduction of CO_2 to fuels and commodities [6,7]. In this case, microorganisms act as an electro-catalyst by taking electrons from the cathode to reduce CO_2. Various multi-carbon products have thus been synthesized from CO_2 [8], acetate being the most frequently obtained (Table 1).

Table 1. Summary of biocathodes reported for reduction of carbon dioxide to acetate and other products.

Cathode material	Polarization (V vs. SHE)	Biocatalyst	Electrode surface (cm^2)	Catholyte volume (L)	Main product	Max production rate ($mM.day^{-1}$)	Max production (mg/L)	Other products detected	Ref
Plain graphite felt	No	Sludge from phototrophic anode	8	0.036	electricity	(750 mW/m^2)	-	-	[9]
Carbon cloth + Pt catalyst	No	*Chlorella vulgaris*	48	0.22	electricity + algae	(5.6 W/m^3)	-	-	[10]
Unpolished graphite sticks	-0.4	*Sporomusa ovata*	65	0.2	acetate	0.8	300	oxo-butyrate	[8]
Unpolished graphite sticks	-0.4	*Clostridium Ljungdahlii*	65	0.2	acetate	0.08	33	oxo-butyrate	[11]
Unpolished graphite sticks	-0.4	*Sporomusa sphaeroides*	65	0.2	acetate	0.04	16	oxo-butyrate	[11]
Chitosan coated carbon cloth	-0.4	*Sporomusa ovata*	47	0.2	acetate	1.07	600	-	[12]
Granular graphite	-0.59	Brewery waste	n.a.	0.075	acetate	4	1710	CH_4, H_2	[13]
Granular graphite	-0.59	Enriched culture from previous MES	n.a.	0.075	acetate	17.25	10500	H_2	[14]
Stainless steel	-0.4	*Geobacter sulfurreducens*	2.5	0.45	glycerol	0.67	800	succinate	[15]
Carbon felt	-0.7 -0.9	Activated sludge	20	0.15	acetate	0.38 2.35	114 n.a	CH_4, H_2	[16]
Carbon felt	-0.750 -0.950	Activated sludge	49	0.245	acetate	0 1.6	0 96	CH_4, H_2	[17]
Nickel nanowire-coated graphite	-0.4	*Sporomusa ovata*	40	0.2	acetate	1.12	540	-	[18]
Graphite plates Anode poised at +0.5 V	Cathode as auxiliary electrode	*Sporomusa ovata*	46	0.25	acetate	0.92	180	-	[19]

Carbon cloth	-0.8	*Clostridium sp.*	9	0.12	acetate butyrate	2.05 0.37	1200 330	ethanol butanol	[20]
Graphite granules	-0.6 -0.8	Enriched culture from brewery	n.a	0.05	acetate hydrogen acetate hydrogen	15 1000 52 1300	5220 - 8770 -	formate butyrate formate butyrate	[21]
Graphite rod	-0.4	Enriched homoacetogenic culture	30	0.5	acetate	9.5	4127	-	[22]
Assembly of graphite felt and stainless steel	-0.9 -0.7	Mixed culture *C.ljungdahlii*	10 15	0.4 0.2	Acetate Acetate	1.3 0.94	630 559	H2 / CH4 Ethanol / H2	[23]

Pure cultures and multispecies inocula have both been shown to be capable of catalysing the electrochemical reduction of CO_2. Among the pure cultures, *Sporomusa ovata* is the most efficient species reported so far. Using a surface-modified carbon cathode polarized at -0.4 V vs. SHE, Zhang et al. [11] obtained 600 mg/L of acetate after 9 days and Nie et al. [18] 540 mg/L of acetate in 8 days. Multispecies inocula have given similar or better performance but it is difficult to compare the various studies reported as they were carried out in different conditions and at different applied potentials. The highest acetate production rate was obtained by Marshall et al. [14], who used granular graphite as the cathode and an enriched culture from a previously established acetogenic biocathode as the inoculum. Their cathodes were polarized at -0.59 V vs. SHE, and rates of acetate production reached 17.25 mM/day with accumulation to 10500 mg/L over 20 days. Hydrogen was also produced by the cathode, at rates reaching 100 mM/d.

The electron transfer (ET) pathway from the cathode to the microbial cells that achieve CO_2 reduction has not been clearly deciphered yet. It has been speculated that microbial cells could gain electrons from the cathode by direct ET through membrane-bound redox systems[6]. Similar direct ET from solid electron donors to microbial species has been identified in natural processes, especially in acidic environments such as mine drainage systems, where oxidation of solid iron (II) and sulfur are dominant microbial activities. For example, *Acidithiobacillus ferrooxidans* is commonly found in deep caves or acid mine drains and thrives in a pH range of 1.5 - 2.5. It has been shown to be able to accept electrons directly from solid Fe(II) minerals (e.g. pyrite) through c-type cytochrome Cyc2 contained in its outer

membrane [24]. Electrons are thus extracted from insoluble minerals and transferred to oxygen, used as the final electron acceptor, which results in minerals being converted to their soluble state.

On the other hand, mediation by hydrogen has also often been suggested. The cathode produces hydrogen by water electrolysis and the microbial species use hydrogen to reduce carbon dioxide to acetate [7,16,17]. In this case, electrosynthesis proceeds in two consecutive steps: firstly, the electrochemical production of hydrogen by water electrolysis and, secondly, the microbial reduction of CO_2, which uses hydrogen.

Actually, microbial reduction of CO_2 to acetic acid using hydrogen as an electron donor is a well-known reaction called homoacetogenic fermentation [25]. First reported by Fischer et al. [26], the discovery was followed by the isolation of the acetogenic strain *Clostridium aceticum* [27], an obligate anaerobic species, which grows either chemolithotrophically with H_2 and CO_2 or chemoorganotrophically with compounds such as fructose, malate or pyruvate. Unfortunately, *C. aceticum* was lost soon after the third paper concerning it was published in 1948 [28]. All attempts to re-isolate a chemolithotrophic acetogen failed until the purification of *Acetobacterium woodii* [29].

In the context of microbial electrochemical conversion of CO_2, it is still difficult to establish whether ET is achieved by a direct pathway or indirectly by homoacetogenic species that use hydrogen produced at the cathode. This is obviously an important fundamental question, the answer to which should considerably impact the way the technology develops toward large-sized industrial equipment.

The purpose of the present work was to assess the possible involvement of the hydrogen route in the microbial electrochemical reduction of CO_2. Two multispecies inocula were used to form microbial cathodes under two different applied potentials: -0.36 and -0.66 V vs. SHE. Both potentials were thermodynamically low enough to ensure CO_2 transformation to acetate, but -0.36 V vs. SHE did not allow hydrogen evolution, while -0.66 V vs. SHE did. Stainless steel was used as the cathode material because it has been shown to be more effective than carbon in achieving fast cathodic ET with microbial cells, particularly with *Geobacter sulfurreducens* [30,31]. The same microbial systems were then implemented in gas-liquid contactors and were fed with hydrogen gas in order to assess their capacity to use hydrogen in the absence of an electrode. Finally, similar hydrogen supply tests were performed with pure cultures of *Sporomusa ovata* to evaluate the capacity of this species to use hydrogen

compared to the performance reported in the literature for the electrochemical process. All these experimental data consistently supported the involvement of the hydrogen route in the microbial electrochemical reduction of CO_2 to acetate. Implementing acetogenic microbial species in purposely designed gas-liquid contactors should now be considered as a relevant way to develop and scale-up the CO_2 conversion systems that have been revealed by microbial electrosynthesis.

2. MATERIALS AND METHODS

2.1 Medium composition

Medium 1 was prepared as already described [30]. It contained: KCl (0.1 g/L), NaH_2PO_4 (0.6 g/L), NH_4Cl (1.5 g/L), and NaHCO3 (2.5 g/L). The solution was sterilized in an autoclave (121°C for 20 minutes) and a trace mineral mix (10mL/L, ATCC MD-TMS) and a vitamin mix (10 mL/L, ATCC MD-VS) were then added.

Medium 2 consisted of medium 1 with the addition of NaCl (45 g/L), $MgCl_2$ (0.1 g/L) and $CaCl_2$ (0.01 g/L).

2.2 Source of microorganisms

Two different environmental samples were used as the inoculum. Biological sludge was collected from a treatment plant (Suez Environnement, Evry, France). Prior to the experiments, the inoculum was acclimated to an H_2 and N_2-CO_2 (80-20%) atmosphere for 5 days at 30°C with the objective of favouring the development of homoacetogenic bacteria. HPLC analyses detected acetic acid at 1980 mg/L and butyric acid at 23 mg/L in the inoculum after the 5 days of acclimation. This inoculum was always implemented with medium 1. The microbial electrochemical reactors were inoculated with 20 mL (3.3% v/v) added into the cathodic compartments. The gas-liquid contactors had 7 mL inoculated into the 210 mL medium.

Sediment collected from a salt marsh of the Mediterranean Sea (Gruissan, France) was used as the second source of microorganisms. This inoculum is known to contain halotolerant electroactive bacteria that have succeeded in forming efficient microbial bioanodes in solutions containing large amounts of salt, such as 45 g/L NaCl [32]. This inoculum was always

implemented with medium 2. The microbial electrochemical reactors were inoculated with 60 mL (10% v/v) in the cathodic compartments and 21 mL was injected into the 210 mL medium of the gas-liquid contactors. HPLC analyses detected lactic acid (370 mg/L), formic acid (91 mg/L) and butyric acid (83 mg/L) in this inoculum.

2.3 Design and operation of microbial electrochemical reactors (MERs)

The microbial electrochemical reactors (MERs) were two-chamber H-shaped electrochemical reactors (Figure 1.A), separated by a 7 cm^2 cation exchange membrane (Fumasep® FKE). A cation exchange membrane was chosen to avoid the migration to the anode compartment of acetate or other anionic compounds possibly produced. The two compartments, made with modified Schott glass (Duran) were of equal volume and dimensions (diameter 101 mm – height 152 mm). Each compartment was filled with 600 mL of medium with a 300 mL headspace. The cathode was a 7 cm * 3 cm stainless steel plate, connected with a 2-mm-diameter screwed titanium wire. The stainless steel electrodes were cleaned with ethanol-acetone mixture 50-50% (v/v), then with a fluoronitric acid solution 2-20%, and finally thoroughly washed with distilled water. The anode was a 15 cm^2 platinum grid, first cleaned by heating to red-hot in a flame. The reference electrode was a saturated calomel electrode (SCE, Radiometer Analytical, +0.241 V vs. SHE). It was placed in the cathode compartment with the tip as close to the surface of the cathode as possible (less than 0.5 cm). Four holes were drilled in the cap that covered each electrochemical compartment; they were used to introduce the electrodes and the tubes for gas bubbling.

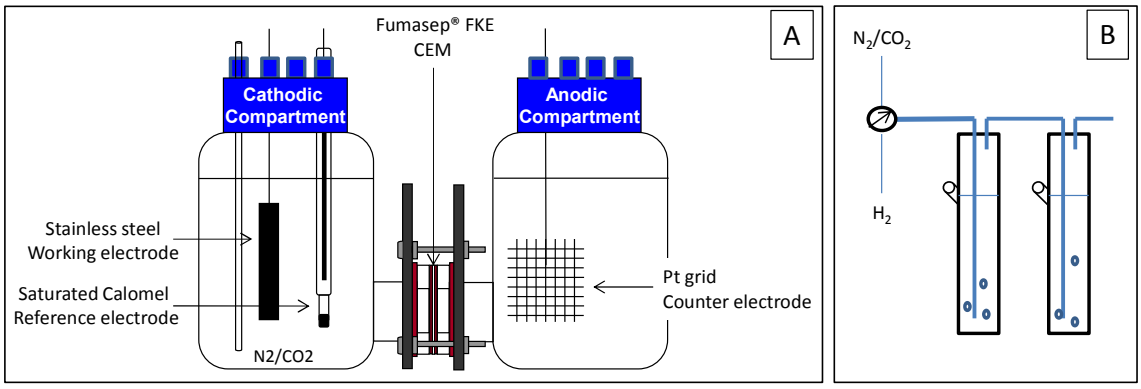


Figure 1. Experimental set-up of (A) the Microbial Electrochemical Reactor - MER and (B) the Gas-Liquid Contactor - GLC

The experiments were conducted under potentiostatic control (chronoamperometry) with a potentiostat (VSP, Bio-logic SA) interfaced with a computer (software EC-Lab). Cathodes

were polarized at -0.60 or -0.90 V vs. SCE, i.e. -0.36 and -0.66 V vs. SHE. The current was recorded every 10 minutes. Chronoamperometry was sometimes interrupted to perform cyclic voltammetry at low scan rate (1 mV/s) starting from the polarization potential and in the range from -0.76 to +0.04 V vs. SHE. All experiments were conducted in a stove thermostated at 30°C. Each compartment was continuously flushed with N_2-CO_2 gas (80-20) to maintain anaerobic conditions. In each case, the pH of the cathodic compartment stabilized at around 7.1. Volumes of 1 mL were sampled from the cathode compartment and filtered at 0.2 µm for HPLC analysis.

Experiments were systematically carried out in duplicate. Four MERs were filled with medium 1 and inoculated with acclimated biological sludge. For two of them, the cathode was initially polarized at -0.36 V vs. SHE for 40 days and then switched to -0.66 V vs. SHE for 26 days. For the other two, the cathode was polarized at -0.66 V vs. SHE from the beginning and for 40 days.

Four additional MERs were filled with medium 2 and inoculated with salt marsh sediment inoculum. Two were run with the cathodes polarized at -0.6 V vs. SHE for 30 days, while the cathodes of the other two were polarized at -0.66 V vs. SHE.

2.4 Gas-Liquid Contactor (GLC)

Experiments without electrodes were run with the same media and inocula in gas-liquid contactors (GLCs) containing 210 mL medium (Figure 1.B). Washing bottles were used with a contact medium column height of 120 mm. The gas feed tube of each contactor was immersed to 7 mm from the bottom and the bubbles came freely out from the outlet of the tube. In the so-called "improved GLCs", the outlet of the gas feed tube was equipped with a porous tube with an aquarium diffuser at the end in order to better sparge the gas into the solution. Solution sampling was possible through a connection placed at a height of 110 mm. N_2-CO_2 gas was mixed with hydrogen before being injected into the contactors. Gas flows were controlled using flow valves (1 valve for N_2-CO_2, 1 valve for H_2 and 1 valve for the mixed gas before its injection into the contactor). GLCs were maintained at 30°C in a water bath.

The media were inoculated and cultures were bubbled with 10 mL/min of N_2-CO_2 (80-20) gas mixed with hydrogen as the electron donor. Duplicate experiments were carried out using two

GLCs in series with the gas outlet of the first bottle being the gas inlet of the second bottle. A total of 12 GLCs were run.

A first experimental run was carried out with 6 GLCs using medium 1 inoculated with acclimated biological sludge, with different hydrogen flow rates. Hydrogen was supplied continuously at a constant flow rate of 2 mL/min in two GLCs and at 6 mL/min in another two. In the last two, hydrogen was alternately supplied at a rate of 2 mL/min for 8 hours followed by 0 mL/min for 16 hours by turning the flow on and off. Each GLC was continuously fed with N_2-CO_2 at 10 mL/min. A second experimental run was carried out in identical conditions using 3 "improved GLC" with a constant hydrogen flow rate of 0.5 mL/min.

Two GLCs were implemented with medium 2 inoculated with salt marsh sediment using 10 mL/min of N_2-CO_2 (80-20) gas mixed with 6 mL/min of hydrogen as the electron donor.

Four GLCs were implemented with pure culture of *S. ovata* (see below).

Initially the pH was stable at around 7.1 in each case but, at the end of the experiment, pH values were measured in a range of 5.5 to 7.3 depending on the amount of acetate produced.

2.5 Culture of *Sporomusa ovata* in GLCs

S. ovata was grown in the DSMZ-recommended growth medium (DSMZ 311) with casitone and resazurin omitted. A volume of 20 mL of the growing cells was used to inoculate the GLCs in the same medium but with the betain omitted. In two GLCs, the culture was continuously fed with an excess of hydrogen-N_2-CO_2 (50-40-10) gas mixture (40 mL/min). Two control experiments were carried out in GLCs fed only with N_2-CO_2 (80-20). Samples were taken every day and filtered at 0.2 µm for HPLC analyses.

2.6 HPLC analyses

Samples were analysed for organic acids, sugar and ethanol by HPLC (Thermo Scientific, France) using a Rezex ROA-Organic acid H+ (8%), 250*4.6 mm phase-reverse column (Phenomenex, France) thermostated at 30°C and associated with a refractive index detector in series with a UV detector. The elution was performed at 170 µL/min with an aqueous solution of sulfuric acid 10 mM (pH 2.2). The column was calibrated with a mixture of formate, acetate, lactate, propionate and butyrate, in the analysis concentration range.

3. RESULTS

3.1. CO_2 electroreduction using acclimated biological sludge as catalyst

The stainless steel cathodes of two identical MERs were polarized at -0.36 V vs. SHE. After 4 days, the cathodic compartments were inoculated with acclimated biological sludge (3.3% vol/vol). During the 40 days of polarization at -0.36 V, current density never exceeded 0.1 A/m^2 and acetate was only detected initially due to the addition of the inoculum containing acetate (Figure 2). When the potential was switched to -0.66 V vs. SHE, current density increased immediately to -1.2 A/m^2 for one reactor and -1.4 A/m^2 for the other. Acetate started to be produced, reaching 98 mg/L and 135 mg/L after 7 days. During this period, the production rate was 132 $mM/day/m^2$ on average. This value was of the same order of magnitude as that obtained by Su et al. [16] working with 20 cm^2 of carbon felt at -0.70 V vs. SHE. They used activated sludge as the inoculum and found a maximum acetate production rate of 187 $mM/day/m^2$, the production of hydrogen also being detected.

Acetate concentration decreased 13 days after the switch in potential. A methanogenic population probably developed in the compartment, consuming the acetate produced. Methane has actually been detected in a number of MERs [13,16,17], especially when an inhibitor (such as bromoethanesulfonate) was not used, as was the case here.

A control experiment run in the same conditions for the same length of time but keeping the electrodes at open circuit (no potential applied) did not produce any acetate ; the only acetate present came from the bacterial injection.

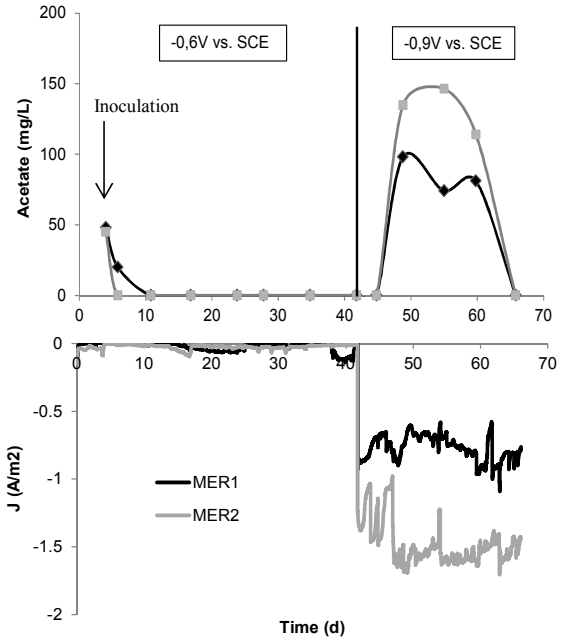


Figure 2. Production of acetate and associated chronoamperometry in MERs initially polarized at -0.36 V vs. SHE for 40 days and then switched to -0.66 vs. SHE. On the 4th day, the MERs were inoculated with acclimated sludge.

A second set of two MERs was run in the same conditions but with an imposed potential of -0.66 V vs. SHE from the beginning (Figure 3). Current densities around -1.5 A/m^2 were recorded immediately at this potential. This confirmed that the immediate increase of current density observed in the previous experiments when the potential was switched from -0.36 to -0.66 V vs. SHE was due to the abiotic electrochemical reduction of water to dihydrogen at the surface of the stainless steel cathode. Bacteria were inoculated 3 days after the start of polarization. Acetate measured just after inoculation corresponded to the acetate contained in the acclimated sludge. In contrast to the previous experiments performed with an initial applied potential of -0.36 V vs. SHE, acetate did not disappear after a few days and its initial concentration was maintained. From day 10, the concentration of acetate increased. Between day 10 and day 20, the acetate concentration increased from 96 ± 3 mg/L to 244 ± 20 mg/L at an average rate of 140 mM/d/m^2 over 10 days, which gave a Faradic yield of 53%. Then, the acetate concentration decreased in both MERs.

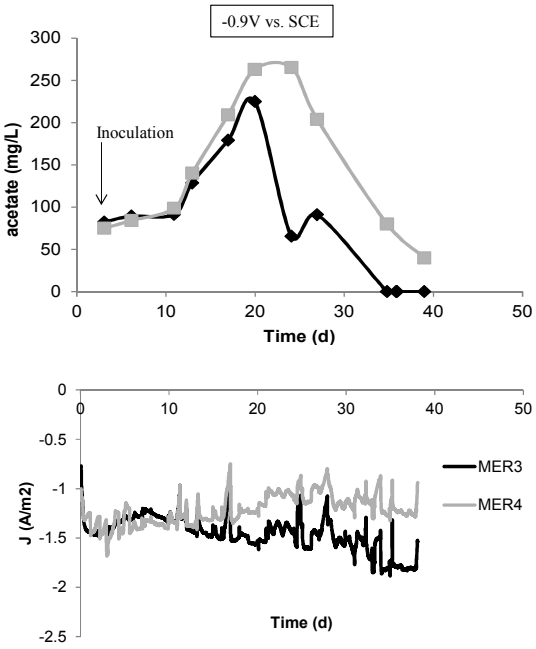


Figure 3. Production of acetate and associated chronoamperometry in MERs polarized at -0.66 V vs. SHE for 40 days. On the 3rd day, the MERs were inoculated with acclimated sludge.

Cyclic voltammetries recorded initially after 1 day of polarization and before inoculation, which was done at day 3, confirmed that there was no hydrogen evolution at -0.36 V vs. SHE but there was hydrogen evolution at -0.66 V vs. SHE. (Figure 4-A). Moreover The CVs recorded during the chronoamperometry (Figure 4-B) showed no change. The presence of microorganisms (sessile and/or planktonic) did not significantly change the behaviour of the electrode.

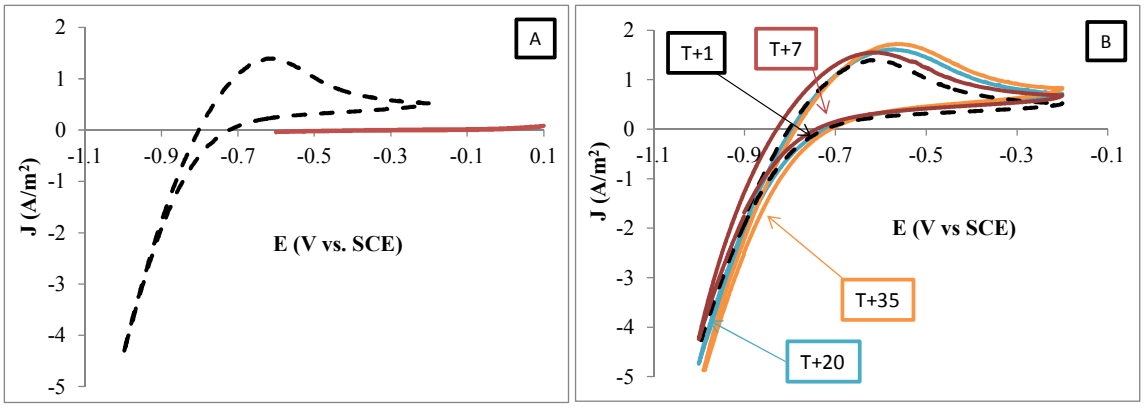


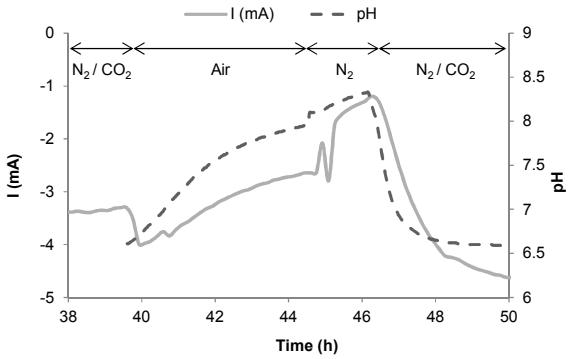
Figure 4. Cyclic voltammetries recorded at 1 mV/s. (A) CV recorded before bacteria injection (abiotic) and after 1 day of polarization (T+1) at-0.36 V vs. SHE (= -0.60 V vs. SCE) solid line and -0.66 V vs. SHE (= -0.90 V vs. SCE) dotted line. (B) CV recorded at days 1, 7, 20 and 35 during the chronoamperometry at -0.66 V vs. SHE.

3.2. CO_2 electroreduction using sediment from a salt marsh as catalyst

Four MERs were started using sediment from a salt marsh as inoculum in a highly saline medium that was supplemented with NaCl 45 g/L. Such high salinity should be a great advantage if the objective is to scale up to large-sized MERs because it allows the internal resistance of the reactor to be significantly decreased in comparison to the low ionic conductive electrolytes that are commonly used in MERs [33]. Two MERs were polarized at -0.36 V vs. SHE, and the other two at -0.66 V vs. SHE.

Current densities never exceeded 0.2 A/m^2 at -0.36 V, whereas they were around 2 A/m^2 from the start of polarization at -0.66 V. The reduction current was established before bacteria were injected, confirming that the electrochemical reaction was the abiotic reduction of water to hydrogen. Acetate, formate or other VFAs were never detected during the 28 days of the experiments in any of the four MERs.

Careful observation of the chronoamperometries revealed variations in current, which were exactly correlated with the fluctuations in CO_2 injection. In the MERs polarized at -0.66 V vs. SHE, when the CO_2 flow rate increased, the reduction current increased almost immediately (absolute value of the current). Specific checks were carried out at the end of the experiments to explain this behaviour. Bubbling air into the reactors instead of N_2-CO_2 continuously decreased the reduction for 4 hours. Actually, the current evolution perfectly fitted the pH increase that was provoked by desorption of CO_2 from the medium during air bubbling (Figure 5). Bubbling pure nitrogen instead of air decreased the reduction current (absolute value) a little more, because it suppressed the reduction of oxygen. Finally, using back N_2-CO_2 made the pH decrease, with the concomitant current recovery. Actually, the variations in current were related to the pH, which was linked to the CO_2 flow rate. An increase in CO_2 flow rate led to acidification of the solution, which favoured the electrochemical reaction of water reduction. The dependence of the current on CO_2 flow rate that was observed here did not indicate that CO_2 was the reactant of the electrochemical reaction; it was an indirect phenomenon due to pH evolution.



317

318 **Figure 5. Evolution of the pH due to different gas injection with or without carbon dioxide gas and the concomitant**
319 **variation in intensity at a stainless steel cathode polarized at -0.66 V vs. SHE.**

320

321 ### 3.3. CO_2 reduction using hydrogen as electron donor with acclimated biological
322 ### sludge as catalyst

323 Since hydrogen was strongly suspected to be the intermediate electron carrier in the
324 experiments conducted with the acclimated biological sludge, further experiments were run
325 without electrodes but feeding the same medium directly with hydrogen, at different gas flow
326 rates. Experiments were performed in gas-liquid contactors (GLCs) with 3 different hydrogen
327 gas flow rates: constant flow rates of 2 mL/min and 6 mL/min and intermittent feeding in
328 cycles of 2 mL/min for 8 hours followed by 0 mL/min for 16 hours. Each reactor was
329 continuously fed with N_2-CO_2 at 10 mL/min.

330 Acetate started to be produced after 2 days of latency in each GLC (Figure 6). The production
331 rate was linked to the hydrogen flow rate. A maximum production rate of 423 mg/L/day (7.2
332 mM/day) was reached between days 2 and 7 with the highest hydrogen flow rate, while the
333 acetate production rate was 244 mg/L/day (4.1 mM/day) for the contactors supplied with
334 hydrogen at 2 mL/min. The intermittent hydrogen supply led to significantly lower acetate
335 production, with a maximum production rate of 78 mg/L/d between days 7 and 14. After 15
336 days, the intermittent hydrogen supply was switched to continuous mode at 2 mL/min. This
337 change led the acetate concentration to increase to the same maximum plateau as in the other
338 reactors. The hydrogen flow rate was consequently a major parameter impacting the acetate
339 production rate.

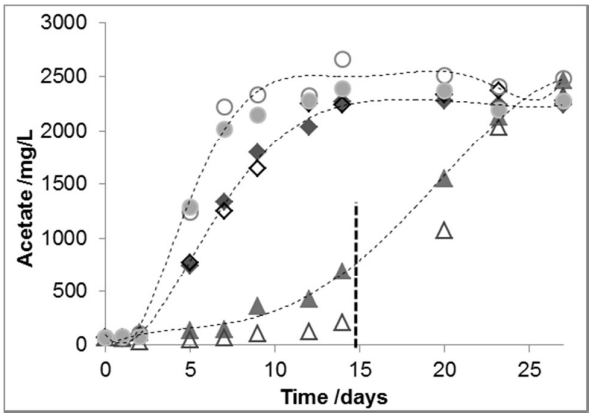


Figure 6. Acetate production in the 6 Gas-Liquid Contactors inoculated with acclimated activated sludge and fed with N_2/CO_2 gas mixed with different H_2 flow rates. H_2 was injected at 6 mL/min in GLCs 5-6 (round), 2 mL/min in GLCs 3-4 (square) and with alternating supply in GLCs 1-2 (triangle) during the first 15 days. The dotted line represents the switch in the feeding mode for GLC 1-2: from the alternating mode to continuous feeding at 2 mL/min. Each reactor was continuously fed with N_2-CO_2 at 10 mL/min

A maximum acetate concentration of around 2500 mg/L was reached after 15 days. This concentration was 10 times higher than those obtained with the bioelectrochemical reactors at -0.66 V vs. SHE.

It was comparable to the acetate levels obtained with a pure culture of the acetogen *Clostridium ljungdahlii* using cysteine as the electron donor [34]. The microbial system implemented here, which consisted of acclimated biological sludge in medium 1, proved to be fully efficient to reduce CO_2 to acetate with hydrogen as electron donor. Moreover, its performance was directly controlled by the hydrogen supply rate in the GLCs. In comparison, the MERs gave lower performance, probably because of the lower hydrogen production rate. The reduction current density around 1.5 A/m^2 recorded during the chronoamperometries at -0.66 V vs. SHE (Figures 2 and 3) corresponded to a hydrogen production rate of 0.02 mL/min. The lower acetate production rates obtained in MERs were consistent with the lower hydrogen supply rate achieved by the cathode compared to that of GLCs.

The hydrogen production rate of 0.02 mL/min and the maximum acetate production rate of 140 mM/d/m^2 gave a hydrogen conversion yield of 53 % for the MERs polarized at -0.66 V vs. SHE (0.17 mmole/d acetate was produced, while 1.29 mmole/d hydrogen was supplied). The same calculations made for the GLCs supplied with 6 and 2 mL/min hydrogen led to hydrogen conversion yields of 1.6% and 2.7 %, respectively (7.2 and 4.1 mM/day production

rates of acetate mean that 1.5 and 0.86 mmole/d of acetate were produced, while 386 and 129 mmole/d of hydrogen were supplied).

The hydrogen conversion to acetate was maximized with the very low hydrogen supply achieved by the cathode in the MER [35]. The electrode was a more efficient hydrogen sparger than the simple tube used in GLCs. The cathode operating at low current density formed very small hydrogen bubbles, which drove a more efficient gas transfer to the liquid than the big bubbles formed at the outlet of the pipe used in GLC. To check this hypothesis, a second run of experiments were performed with three "improved GLCs", which were aimed at ensuring more efficient hydrogen sparging into the solution. With a hydrogen supply rate of 0.5 mL/min, a maximum acetate production rate of 5.2 mM/day (309 mg/L/day) was maintained for around five days (Figure 7). The yield of the conversion of hydrogen to acetate was 13% (1.1 mmole/d acetate was produced, while 32.1 mmole/d hydrogen was supplied). Changing the gas sparger in the GLCs improved the yield of hydrogen conversion to acetate by a factor of 8. These results confirmed that hydrogen gas/liquid transfer is one of the main parameters to be optimized when scaling-up microbial conversion of CO_2 to acetate.

The fair results recorded with the MERs with respect to hydrogen conversion yield are probably linked to the low hydrogen supply and the efficient gas sparging achieved by the cathodes. Obviously, the occurrence of direct electron transfer may be another reason for the better electron recovery in MERs but deciphering the fine electron transfer mechanisms was not the purpose of the present study. Here, it was shown that the cathode abiotically produced twice as much hydrogen as needed to sustain the acetate production. The same inoculum implemented in identical conditions with direct hydrogen supply in GLCs gave similar acetate production with higher rates but lower hydrogen conversion yields. The GLC experiments confirmed that, the hydrogen yields were strongly linked to the gas sparger efficiency. These results showed that the hydrogen route plays an important role in the electro-microbial conversion of CO_2. Gas/liquid technology should consequently open up an alternative way to scale-up the systems discovered in the field of microbial electrosynthesis.

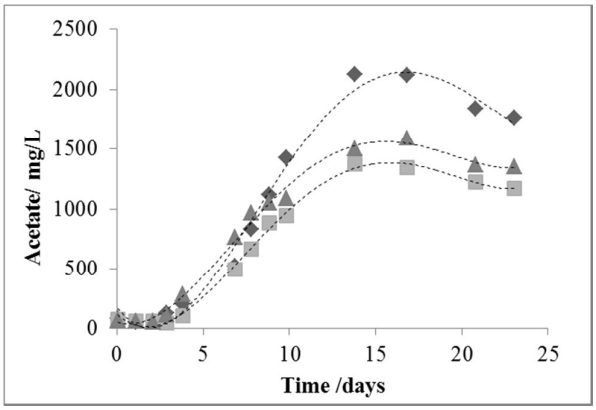


Figure 7. Acetate production in 3 improved Gas-Liquid Contactors (triplicate) inoculated with acclimated activated sludge and fed with H2 at 0.5 mL/min and N_2-CO_2 at 10 mL/min.

3.4. CO_2 reduction using hydrogen as electron donor with sediment from salt marsh as catalyst

Similar hydrogen feeding was attempted with the second microbial system used here: salt marsh sediment implemented in the highly saline medium 2. Hydrogen was supplied at a flow rate of 6 mL/min. Formate was the first molecule to be produced, after around 2 days of latency (Figure 8), with a maximum production rate of about 200 mg/L/d on the first day of production. An average maximum formate concentration of 380 mg/L was reached after 10 days, while acetate started to be produced after 13 days, concomitantly with the decrease of formate concentration.

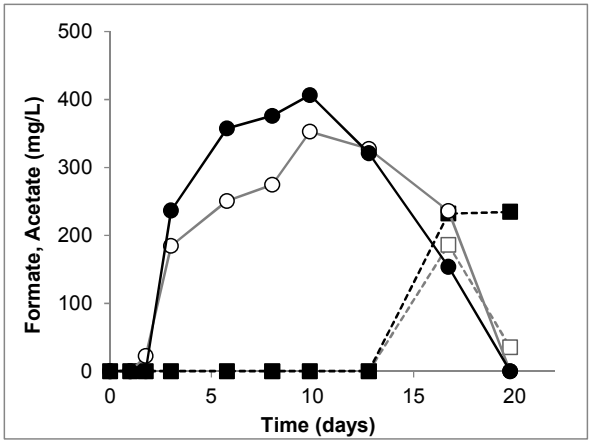


Figure 8. Formate (round) and acetate (square) production in 2 Gas-Liquid Contactors inoculated with salt marsh sediment and fed with 10 mL/min N_2.CO_2 gas mixed with 6 mL/min H_2.

Formate can be produced by enzymatic reduction of CO_2 in an NADH- or ferredoxin-dependent manner [36]. Moreover, formate was previously found to be a precursor of the methyl group of acetate in *Clostridium sp.* [37], which would explain the concomitance of formate consumption with acetate production.

The experiments performed with direct hydrogen supply showed that the microbial system based on salt marsh sediment was significantly less efficient than the acclimated biological sludge. The electrochemical route led to considerably lower performance than direct hydrogen supply with the biological sludge inoculum. As the salt marsh sediment was less efficient than biological sludge, it was not surprising that no production was found with the salt marsh sediment inoculum in the electrochemical reactors. Actually, direct hydrogen supply succeeded in revealing the capability of weakly efficient microbial systems to reduce CO_2.

3.5. CO_2 reduction coupled to hydrogen oxidation using *Sporomusa ovata*

The model microorganism *Sporomusa ovata*, which is known for its electrosynthesis ability [8], was also implemented with direct hydrogen supply in gas-liquid contactors. In the pre-culture, it was noticed that the bacteria were not able to grow without yeast extract, but yeast extract may be a source of electron donor(s), which can support the reduction of CO_2. So yeast extract was kept in the medium and experiments with and without hydrogen supply were carried out in parallel in order to measure the role of hydrogen (Figure 9). Cultures were run in duplicate.

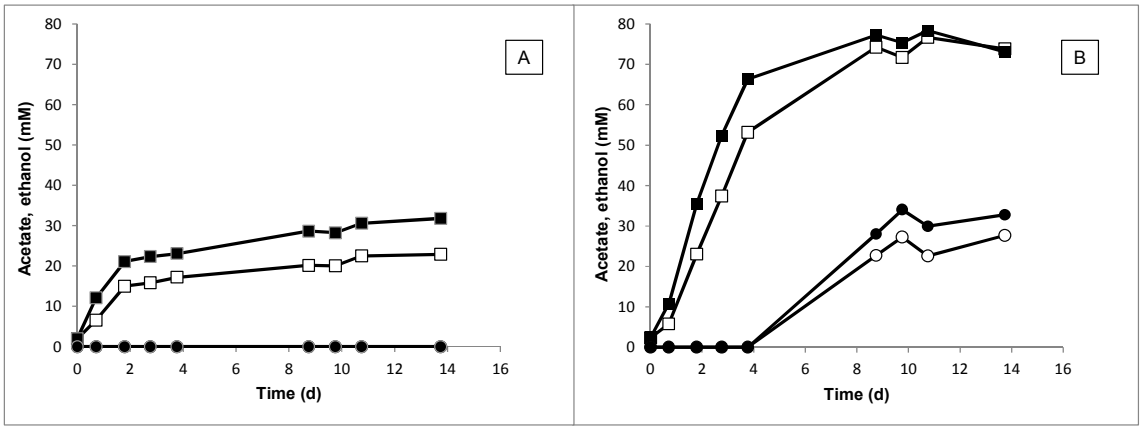


Figure 9. Acetate (square) and ethanol (round) production in GLCs inoculated with *Sporomusa ovata* in its specific medium containing yeast extract (YE) fed with N_2/CO_2 gas (20 mL/min), without hydrogen supply (7.A) and with hydrogen supply injected at 20 mL/min (7.B).

When no hydrogen was injected (Figure 9.A), up to 1638 ± 270 mg/L of acetate was produced in 14 days, with the largest part produced during the first 2 days. When hydrogen was supplied (Figure 9.B), production of acetate was almost tripled, to 4542 ± 90 mg/L in 9 days. Moreover, ethanol started to be produced after around 7 days, when acetate production reached a plateau. Production of 1411 ± 156 mg/L of ethanol was obtained after 10 days of culture.

The difference observed with and without hydrogen supply corresponded to a production of acetate of up to 2904 mg/L at a maximum rate of 867 mg/L/day (14.7 mM/day) during the first 3 days. Moreover, ethanol production was promoted with hydrogen supply. The product ratio of ethanol and acetate was 0.49 g ethanol per gram acetate.

4. DISCUSSION

4.1 Discussion of the experimental results

From a thermodynamic point of view, the electrochemical reduction of carbonate ions HCO_3^- to acetate:

$$2\ HCO_3^- + 9\ H^+ + 8\ e^- \Leftrightarrow CH_3COO^- + 4\ H_2O \quad (1)$$

is possible at potentials less that the formal potential of the equilibrium ($E^{0'}_{CO2/acetate}$):

$$E^{0'}_{CO2/acetate} = E^{0}_{CO2/acetate} - \frac{RT}{nF}\ln\frac{[CH_3COO^-]}{[HCO_3^-]^2[H^+]^9} \quad (2)$$

where $E^{0}_{CO2/acetate}$ is the standard potential of the CO_2/acetate pair (0.187 V/SHE [38]), R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature (303 K), n = 8 is the number of electrons exchanged, F is the Faraday constant (96,485 C mol^{-1}), [ ] are the concentrations (mol L^{-1}), as activities were taken to be equal to concentrations because of their low values. pH of the solution was 7.0. The reactors were supplied with N_2-CO_2 80:20, i.e. a CO_2 partial pressure of 0.2 atm. The concentration of the HCO_3^- ions calculated assuming equilibrium with CO_2 with pK = $10^{-6.3}$ gave 1.03 mM, so that:

$$E^{0'}_{CO2/acetate} = 0.143 - 0.067\ pH - 0.0075\ \log([CH_3COO^-]) \quad (3)$$

The value of the acetate concentration produced did not have a significant effect on the formal potential, which was -0.298 V vs. SHE with an acetate concentration of 0.2 mM (11.8 mg/L) and decreased only to -0.309 V vs. SHE for 5 mM (295 g/L). The maximum acetate concentration produced in the MERs never exceeded 5 mM. It can thus be concluded that the applied potential of -0.36 V vs. SHE was thermodynamically appropriate to support the transformation of HCO_3^- to acetate and it was not a cause of the limitation of this production to less than 5 mM.

Hydrogen evolution at neutral pH:

$$2\ H_2O + 2\ e^- \Leftrightarrow H_2 + 2\ OH^- \quad (4)$$

has a formal potential expressed as :

$$E^{0\prime}{}_{H2O/H2} = E^{0}{}_{H2O/H2} - 2.3\frac{RT}{2F}\{2\ (pH\text{-}14) + \log p_{H2}\} \quad (5)$$

where $E^0{}_{H2O/H2}$ is -0.828 V/SHE and p_{H2} is the hydrogen partial pressure. Assuming that hydrogen evolved at 1 atm gave the final equation:

$$E^{0\prime}{}_{H2O/H2} = 0.014 - 0.060\ pH \quad (6)$$

At pH 7.0, hydrogen can start to evolve from -0.41 V vs. SHE.

The applied potential of -0.36 V vs. SHE was too high to allow hydrogen gas evolution and acetate production was never observed at this potential, although it was thermodynamically possible. In contrast, the potential of -0.66 V vs. SHE allowed hydrogen evolution and led to significant production of acetate when sludge was used as the inoculum. Furthermore, the same inoculum produced larger amounts of acetate and displayed higher production rates when it was directly fed with hydrogen in gas-liquid contactors than when it was in a MER.

From a fundamental point of view, it is difficult to compare a heterogeneous catalytic process, which is controlled by the surface area of the solid catalyst, with a gas-liquid reaction, which generally depends on the gas/liquid interface area. The direct comparison of the volumetric production rates obtained in the MERs with those in the GLCs (here 0.29 mM/d and 7.2 mM/d, respectively) is useful for a quick, preliminary comparison but cannot constitute an appropriate basis for envisioning the performance of larger sized devices. The MER

performance is directly linked to the "cathode surface area vs. solution volume" ratio (the cathode of 21 cm^2 surface area in 0.6 L gave 3.5 m^2/m^3). The rather small surface area of 21 cm^2 chosen here was the result of a compromise. On the one hand, according to electroanalysis rules, the cathode must be as small as possible in order to determine the electrochemical kinetics in the absence of most possible limitations [39] but, on the other hand, the production must be sufficient to allow the concentrations of the products to be measured easily with acceptable accuracy. This choice led to modest volumetric production rate but to allow the rate per unit surface to be evaluated in optimal conditions. The rates per unit surface determined in this condition of 0.084 mole/d/m^2 (140 mM/d/m^2 in 0.6 L) can thus be used to design an appropriate electrochemical reactor. The following calculation gives a basis for comparing a GLC with the MER that could achieve the same acetate production in 1L volume. In GLCs, the biological sludge inoculum ensured a production rate of acetate of 7.2 mM/d. To have 1L, the GLC could be straightforwardly scaled up to a column of 5 cm diameter and 51 cm height of liquid. Actually, the chosen diameter here is the same as that of the GLCs used in the present study, only the height was adjusted to correspond to 1 L volume of solution. To achieve the same production rate of 7.2 mM/d, the MER needs a cathode surface area of 857 cm^2 (7.2 / 84 m^2). With conventional filter-press architecture, the MER could be composed of two cells each equipped with a cathode 10 cm wide and 43 cm long. The distance between the cathode and the anode has to be as short as possible to minimize the ohmic drop, particularly because of the low ionic conductivity of the solution used here in comparison to the electrolytes used in conventional electrochemical cells (see section 4.2.3). With 5 mm between the cathode and the membrane, the MER would contain approximately 430 mL, so a closed loop equipped with a pump and a storage tank of approximately 570 mL volume would need to be connected to the cathodic compartment of the MER (actually the volumes of the tubes and other side volumes should be subtracted).

This example points out the technical complexity of the MER in comparison to the GLC technology. Firstly, the MER architecture is technically complex: two electrodes to be maintained as close as possible with a separator between them, perfect tightness of each compartment, separate tubing for the cathode and anode compartments, connection of several cells, etc. In addition, the electrochemical process control can raise difficulties, particularly if microorganisms are included inside: ionic transfers between the two compartments must be managed in order not to affect microbial growth, pH gradients [40] must be controlled, disturbance of the anaerobic conditions of the cathode compartment by the oxygen evolving at

the anode must be avoided, etc. The chemically rich culture media that are used in microbial electrosynthesis and the presence of microorganisms in the cathode compartment may also induce membrane (bio-)fouling. All these hindrances could certainly be overcome, at the price of finding compromises and fine tuning the operating conditions, but they give the MER a huge level of complexity in comparison with the GLC technology.

According to the data obtained here with the biological sludge inoculum, the main advantage of the MER might be thought to be the Faradic yield of around 53%, which means that about 53% of the hydrogen produced is used to produce acetate. The yield of hydrogen conversion to acetate was low in GLCs, 1.6% to 2.7%, because no effort had been made to save hydrogen. Simply improving the sparger with means available in the laboratory increased the hydrogen conversion yield to 13%. Gas-liquid transfers are well mastered at industrial level and chemical engineering offers many solutions to further improve the rough GLCs used here (see section 4.3).

Salt marsh sediment was not an adequate inoculum for MERs. Nevertheless, direct supply with hydrogen in GLCs revealed its capacity to produce acetate. The maximum production rate was around 5 times lower than with sludge. Salt marsh sediment was a less efficient inoculum than the biological sludge. Because of the lower production rate of hydrogen in the MER, the electrochemical process was not able to exploit the homoacetogenic capacity of this inoculum but GLC offered a simple way to reveal the inoculum capacity.

Finally, the experiments performed with pure cultures of *Sporomusa ovata* revealed the full interest of GLC technology in the context of autotrophic CO_2 reduction. Several studies have been reported in the literature, implementing *S. ovata* in electrochemical reactors with the electrode as sole electron donor (absence of yeast extract and hydrogen)[8,12,18]. To the best of our knowledge, the highest reported production was 600 mg/L [12] and the maximum production rate was 1.12 mM/d [18]. Here the maximum acetate concentration and production rate were around 5 times (2904 mg/L) and 13 times (14.7 mM/day) higher than the results reported in MERs.

The GLCs revealed the capability of *S. ovata* to produce ethanol at concentrations up to 35 mM, which, to the best of our knowledge, has never been obtained before. *Sporomusa ovata* was known to exhibit fermentative properties, as is typical of acetogenic bacteria, with the

production of a large amount of acetate. However, only a small amount of ethanol (below 1mM) has been reported to be produced so far [41]. For comparison, Younesi et al. [42] obtained a maximum concentration of ethanol of 600 mg/L (13 mM) using *Clostridium ljungdahlii* grown on syngas. Unlike *S. ovata*, *Clostridium ljungdahlii* was already identified as an acetogen that produced ethanol from CO_2 [23,43]. Actually, a few acetogens as *C. ljungdahlii*, *C. autoethanogenum* or *C. ragsdalei* are able to form large amounts of ethanol from CO_2. Very recently, metabolic schemes have been proposed to elucidate how these anaerobes conserve energy, by determining the specific activities and cofactor specificities of all relevant oxidoreductases in cell extracts of H_2/CO_2 -grown *C. autoethanogenum* [44].

Here, *S. ovata* implemented with a direct supply of hydrogen gas revealed an interesting capacity to produce ethanol at a higher level than species already identified as ethanol producers. This unexpected result is another illustration of the technological interest of the GLC procedure to develop microbially-catalysed CO_2 reduction in value added molecules.

The three kinds of results, obtained in the present study with two environmental multispecies inocula and pure cultures, indicate direct hydrogen supply with a gas-liquid contactor as a valuable strategy to exploit the hydrogen route of CO_2 reduction. It should be noted that the possibility of direct electron transfer from cathodes to microbial biofilms in the absence of hydrogen as an electron carrier is not in doubt but, simply, it was not the subject of this study, which aimed to show that similar results can be obtained with GLC but in a technologically simpler manner. In parallel to the fundamental studies that aim to decipher the fine electron transfer mechanisms, the results described here showed that the hydrogen pathway should now be considered as a promising route that could be implemented at large scale via dedicated technologies.

4.2. Why implement the hydrogen route in gas-liquid contactors

To be economically efficient, an electrochemical reactor must operate at high current densities. For example, chlor-alkali cells work at 1 500 to 3 000 A/m^2, the electrosynthesis of adiponitrile from acrylonitrile is performed at 2 000 to 4 500 A/m^2 and water electrolysis is carried out at current densities above 1 000 A/m^2 in conventional cells and up to 10 000 A/m^2 in bipolar configurations [45]. As soon as the objective is to design a large-sized industrial process, an electrochemical process requires complex technology. Sophisticated technical

solutions must be implemented to solve elementary problems such as current collection on the electrodes, perfect sealing of the different parts (electrodes, membrane, and frames), control of the fluid motion in the narrow electrode-membrane spaces, electrical and hydraulic connections of several cells, etc. All these issues quickly become technically very cumbersome as the surface area of the electrodes increases. This is the reason why electrochemical processes are envisioned for large-scale production only when high current densities can be ensured.

4.2.1. Direct electron transfer in the absence of hydrogen evolution in the context of industrial constraint (Figure 10.A)

The formal potentials for the CO_2/acetate and H_2O/H_2 redox couples were -0.30 and -0.41 V vs. SHE at pH 7.0. These values are not considerably affected by the concentrations of the reactive and product compounds (Equations (3) and (6)), so the difference between the two redox couples is of the order of 100 mV in common operating conditions. Direct electron transfer for CO_2 reduction to acetate can start at -0.30 vs. SHE, while hydrogen evolution rapidly gives high current density when the potential becomes more negative than -0.41 V vs. SHE. CO_2 reduction through direct electron transfer could be exploited inside this narrow potential zone, approximately 100 mV wide, in order to remain above the domain of hydrogen evolution. The overpotential of 100 mV is too small to ensure high current density for CO_2 reduction via direct electron transfer without penetrating the domain of hydrogen evolution. The ideal answer to the scientific challenge of exploiting the direct electron transfer pathway at industrial scale would be to design electrode materials that accelerate electron transfer to the biofilm while slowing the kinetics of hydrogen evolution. This would be an elegant solution for developing microbial electrosynthesis based on direct electron transfer. Nevertheless, in the current state of the art, designing such electrode materials remains a tremendous challenge, which still requires deep fundamental research.

The difficulty of implementing the direct electron transfer zone with high current density is straightforwardly linked to the proximity of the formal potential of the conversion of CO_2 to acetate with that of hydrogen evolution. The same situation is encountered for the conversion of CO_2 to ethanol:

$$2\ HCO_3^- + 14\ H^+ + 12\ e^- \rightarrow CH_3CH_2OH + 5\ H_2O \quad (E'_0 = -0.335\ V\ vs.\ SHE\ at\ pH\ 7.0) \qquad (7)$$

but would be less stringent for the conversion of acetate to ethanol:

618 $2\ CH_3COO^- + 6\ H^+ + 5e^- \rightarrow CH_3CH_2OH + 3\ H_2O$ (E'_0 =-0.212 V vs. SHE at pH 7.0) (8)

619 The conclusion may be completely different for reactions with formal potentials farther from
620 that of hydrogen evolution. For example, the conversion of CO_2 and succinate to glycerol,
621 with a formal potential of 0.06 V vs. SHE at pH 7.0 [15] offers a possible overpotential range of
622 more than 400 mV before reaching hydrogen evolution. High current densities have been
623 reported at potentials up to -0.09 V vs. SHE, at which hydrogen evolution cannot be
624 suspected. This reaction may be appropriate to produce high current density via the direct
625 electron transfer pathway and illustrates the need for further investigations to decipher and
626 then exploit the direct electron transfer pathways for electrosynthesis purposes.

627 *4.2.2. Direct electron transfer coupled to gentle hydrogen evolution in the context of*
628 *industrial constraint (Figure 10.B)*

629 As a second option, it might be envisioned to implement hydrogen evolution and direct
630 electron transfer through the biofilm concomitantly. Both routes could be implemented
631 simultaneously. Direct electron transfer could occur on colonized patches of the electrode
632 surface, while hydrogen could gently evolve on other parts (Figure 10.B). This option might
633 be particularly appealing as some components of the biofilm can catalyse hydrogen evolution.
634 For example, hydrogenases adsorbed on an electrode surface are known to catalyse the
635 reduction of proton/water [46,47] and this catalysis has recently been shown to occur also with
636 hydrogenase released from cells during routine culturing[48]. Obviously, such mechanisms have
637 great importance in the context of microbial corrosion[49,50], where even modest current
638 densities can lead to huge economic losses. Nevertheless, this option does not allow the high
639 current densities required in large-sized industrial plants to be reached. Actually, at high
640 current density, hydrogen evolution would become largely dominant and gas evolution would
641 mechanically keep the microbial cells away from the electrode surface. Vigorous hydrogen
642 evolution from the electrode towards the bulk would obviously preclude colonization of the
643 electrode surface by the microbial biofilm. It has already been observed that hydrogen
644 evolving at the cathode, even under gentle current densities around 10 A/m^2, limits the
645 biofilm formation[23]. In such condition, the benefit of the direct electron transfer route would
646 be annihilated and CO_2 conversion would be driven by the hydrogen route only.

647 *4.2.3. The hydrogen route in the context of industrial constraints (Figure 10.C)*

Finally, in a third option, the electrochemical reactor might be considered as the hydrogen supplier for the bacteria that develop in the bulk. In other words, this third option would consist of introducing the bacteria into a water electrolysis cell in order to perform both hydrogen production and the homoacetogenic CO_2 conversion in the same device. High current densities could be used to support strong hydrogen evolution. The Faradic yields would be very low because of the short residence time of hydrogen in the reactor. Actually, designing efficient water electrolysis cells, i.e. cells able to ensure very high hydrogen evolution rates, but with long hydrogen residence time presents two opposing constraints from an engineering point of view. Other antagonistic requirements would also arise, such as the necessity to work at pH around neutrality for bacterial growth while optimum water electrolysis cells use extremely alkaline solutions (KOH more than 20%, pH 14 and above). The chemical complexity of the culture media can be another hindrance to the electrochemical process, because numerous cations that are necessary to microbial growth (e.g. Ca^{2+}, Mg^{2+}…) are most likely to deposit on the cathode surface due to local alkalinization of the interface. Electrochemical cells also require electrolytes of very high ionic conductivity to keep ohmic power losses to the minimum. Concentrated potassium hydroxide solutions have ionic conductivities above 20 S/m. In contrast, the most common culture media used in microbial electrochemistry have ionic conductivities ranging from 0.5 to amaximum of 2 S/m[32]. For instance, an electrochemical cell with 5 mm inter-electrode distance operated at 1000 A/m^2 must overcome an ohmic drop of less than 250 mV if a conventional electrolyte with ionic conductivity greater than 20 S/m is used, while the ohmic drop would be 5 V with a culture medium of 1 S/m conductivity.

In summary, implementing the acetogenic microbial reaction inside a water electrolysis cell would raise a huge number of cumbersome antagonistic constraints. They would have to be solved at the price of drastic performance degradation with respect to the current level of industrial water electrolysis devices.

In the current state of the art of microbial electrosynthesis, as far as reductions with formal potential not very different from that of hydrogen evolution are concerned, the best strategy for short- or mid-term scaling up is to connect a microbial gas-liquid contactor downstream of a conventional water electrolysis cell. This system constitutes a hybrid system, according to the terminology proposed recently for microbial electrochemical technologies [51]. The high performance of water electrolysis is thus preserved and the efforts to be made for scaling up the homoacetogenic microbial synthesis are focused on the GLC. In this way, the chemical

composition of the culture medium can be fitted to microbial requirements without any concern about possible deposits on the electrode surface or too weak ionic conductivity. The main problem linked to the low microbial reaction rate can be coped with in GLC without the additional constraints due to the electrochemical process.

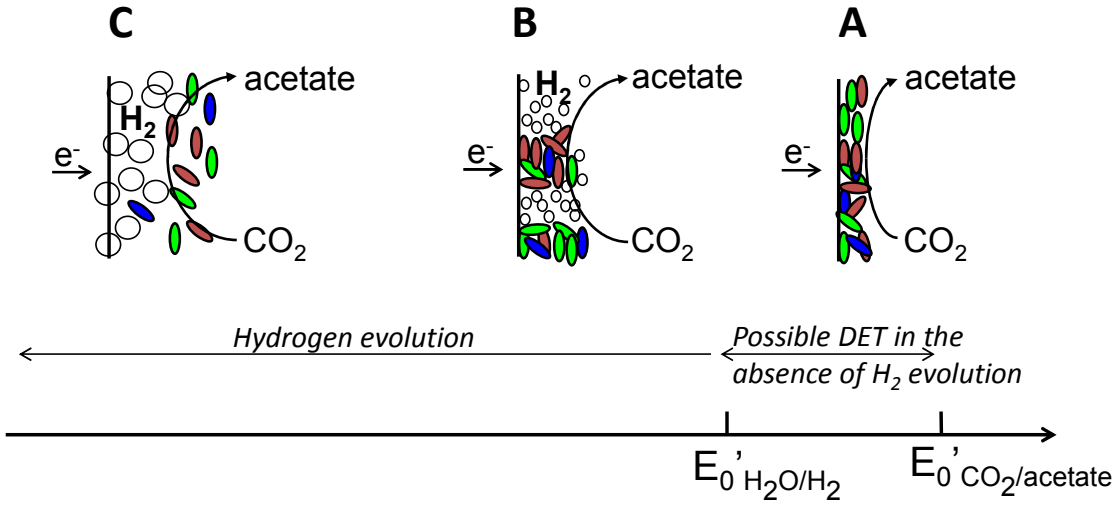


Figure 10 : Scheme of the different potential zones relating to the conversion of CO_2 to acetate vs. hydrogen evolution. A) direct electron transfer can occur in the absence of hydrogen evolution; B) direct electron transfer can occur in biofilm patches concomitantly to hydrogen evolution; C) strong hydrogen evolution precludes colonization of the electrode surface

4.3 How to improve the GLC technology for homoacetogenic CO_2 conversion

The preliminary experiments described here have evidenced the potential of the GLC option and illustrated the fast progress that can be made thanks to simple technological improvements. Here, just changing the gas sparger multiplied the hydrogen conversion yield by a factor of 8. At the industrial level, efficient solutions exist to improve the hydrogen/liquid transfers. Hydrogenation is the most ubiquitous reaction in the commercial organic chemical industry [52] and is commonly implemented in several-ton industrial plants under hydrogen pressure of several bars with metallic catalysts[53].

Here, the microbial catalysis of the conversion of CO_2 to acetate using hydrogen:

$$2\ HCO_3^- + 4\ H_2 + H^+ \Leftrightarrow CH_3COO^- + 4\ H_2O \quad (9)$$

does not ensure such high reaction rates as the metallic catalysts in conventional hydrogenations. That is why the existing technologies need to be adapted to implement the

appropriate hydrogen/liquid transfer. Work in this direction has already started with success, using pure cultures of *Acetobacter woodii* for example, which have ensured a final acetate concentration of 44.7 g/L by working under pressurized H_2:CO_2; more than 50 g/L acetate have been reached in less than 4 days with recombinant strains [54].

Similar studies carried out in the field of carbon monoxide fermentation are also very encouraging. Synthesis gas is a mixture of CO and H_2 (also called syngas), large amounts of which can be obtained by the gasification of biomass (straw, wood, etc.). Syngas fermentation by acetogenic species has started to raise commercial interest for its capacity to produce fuels and chemicals [55]. The low solubility of CO and H_2 has been overcome by chemical engineering solutions [56] so the process has become relatively mature and commercial scaling up can now be reasonably contemplated [57]. The low solubility of CO_2 should be overcome by similar technological solutions. For example, the bubbleless technologies using membrane contactors, which have been patented for syngas fermentation [56] should be a promising way to adapt to the CO_2 conversion routes coming from microbial electrosynthesis.

CONCLUSION

Chronoamperometries performed at two different potentials showed that the hydrogen route was largely involved in the reduction of CO_2 to acetate. Using the same media in gas-liquid contactors supplied with hydrogen led to higher production rates and higher maximum concentrations than the electrochemical reactors. Moreover, gas-liquid contactors revealed a higher capacity of *Sporomusa ovata* to reduce CO_2 than observed so far and also its unsuspected ability to produce ethanol. The autotrophic culture of acetogens in hydrogen-supplied gas-liquid biocontactors should now be considered as a route of great interest for scaling up the CO_2 reduction systems revealed by microbial electrosynthesis.

Acknowledgements

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